

**Systematic Studies of Korean Rodents: III.
Morphometric and Chromosomal Analyses of Striped Field Mice,
Apodemus agrarius chejuensis Jones and Johnson, from Jeju-Do**

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한국산 설치류의 계통분류학적 연구: III. 제주도산 등줄쥐
(*Apodemus agrarius chejuensis* Jones and Johnson)의
형태적 및 염색체 분석

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적 요

한국산 설치류 중 등줄쥐(*Apodemus agrarius*)의 분류학적 연구를 위해서 제주도산 등줄쥐(*Apodemus agrarius chejuensis*)를 채집하였으며 형태적 및 염색체 분석을 한반도 내의 등줄쥐(*A. agrarius coreae*)와 비교 실시하였다. *A. agrarius coreae*와 *A. agrarius chejuensis*는 염색체 핵형에서는 같았으나, 형태적 형질에 있어서는 전자는 작은 형이었으며 후자는 큰 형이었다.

Key words: Systematics, rodent, morphometric, chromosomal, *Apodemus*, Jeju-Do.

INTRODUCTION

Most taxonomic works on Korean rodents were based on the description of pelage colour and external measurements of type specimens (Woon, 1967). The genus *Apodemus* is a fairly distinctive genus of about 12 species, confined to the Palaearctic and northern part of the Oriental regions. It is generally agreed that *A. agrarius* Pallas (striped field mice) is a distinct species, inhabiting from West Germany to Korea. Most of subspecies were designated based on slight differences in pelage colour and/or mean body size (Corbet, 1978).

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Thomas (1906) described striped field mice from the Korean peninsula and Jeju-Do as *Apodemus agrarius coreae*. Jones and Johnson (1965) reported that four subspecies of *A. agrarius* were recognized in Korea: *A. agrarius manchuria* in the extreme northern part, *A. agrarius coreae* throughout the major portion of the peninsula, *A. agrarius pallescens* in the costal lowlands of southern and southwestern Korea, and *A. agrarius chejuensis* in Jeju-Do. They also stated that subspeciation of *A. agrarius* in the Korean mainland was not clearly defined.

Recently, the wide use of computers, the refinements of methods for chromosomal analyses, and new techniques for electrophoretic studies have enabled systematists to interpret an evolutionary process through different lines of research, i.e., chromosomal, phenetic, and molecular.

The role of chromosomal rearrangements in speciation has been intensively studied and discussed (Jackson, 1971; Wilson *et al.*, 1975; Bush *et al.*, 1977; White, 1982). In the genus *Apodemus*, it was concluded by the chromosomal analysis with Korean field mice that samples from Mungyong were not *A. speciosus peninsulae* but *A. peninsulae peninsulae* (Koh, 1986a). In chromosomal analysis with specimens of *A. agrarius coreae* from ten localities it was found that diploid number is 48 with chromosomal complement of 40 acrocentric and 8 metacentric chromosomes (Kang and Koh, 1976; Koh, 1982).

Equal weightings and overall similarity seem inapplicable in defining higher categories above the species level (Farris, 1966). On the other hand, Flake and Turner (1968) noted that the numerical approach offers potential for the resolution of taxonomic problems for populations at infraspecific level. Furthermore, age variation, secondary sexual dimorphism, and geographic variation of morphometric characters should be analyzed before specific status is considered (Mayr, 1969).

Evident age variation and no significant secondary sexual variation were found from univariate and multivariate analyses of morphometric characters of *Apodemus agrarius coreae* from Chongju (Koh, 1983). Based on morphometric analyses with the samples of the same age class in *A. agrarius coreae* from four localities, it was concluded that they were similar with one another enough to be grouped into a single subspecies (Koh, 1985). In a phenetic analysis with the specimens of three subspecies of *A. agrarius* from ten localities, including two localities in *A. agrarius chejuensis*, it was found that *A. agrarius pallescens* is a synonym of *A. agrarius coreae* and that *A. agrarius chejuensis* is different from *A. agrarius coreae* (Koh, 1986b).

The objective of this paper is to analyze karyotype of *A. agrarius chejuensis* in order to compare with that of *A. agrarius coreae* and is to analyze morphometric characters with the specimens from 15 localities in order to confirm that *A. agrarius coreae* is distinct from *A. agrarius chejuensis*.

MATERIALS AND METHODS

Materials

All 292 samples of striped field mice from 15 localities (55 samples of *Apodemus agrarius chejuensis* from seven localities and 237 of *A. agrarius coreae* from eight localities) were trapped and prepared for phenetic analyses (for details see Table 1). Five samples of *A. agrarius chejuensis* from Sanchondan (2♂: K-257, K-259; 3♀: K-275, K-0545, K-270) and one of *A. agrarius coreae* (♂: K-271) from Chongju were also used for chromosomal analyses.

Table 1. Specimens used.

Subspecies name	Locality	No. of specimen
<i>Apodemus agrarius chejuensis</i>	Mosulpo	14
	Jeju	6
	Sanchondan	19
	Yongsil	11
	Saehwa	2
	Jungmun	2
	Bobhochon	1
<i>Apodemus agrarius coreae</i>	Chongju	131
	Mt. Weolak	26
	Mt. Taebaek	19
	Mt. Palgong	21
	Mungyong	22
	Anmyon Island	6
	Kunsan	8
	Mokpo	4
Total	2	15
		292

Samples were collected with live traps and specimens for chromosomal analyses were kept alive for a few days in cages before chromosomal analyses were conducted. Skins and skulls of all specimens are in the collection of the author, Department of Biology, College of Natural Sciences, Chungbuk University, Chongju, Korea.

Chromosomal analyses

The bone-marrow *in vivo* method by Ford and Hamerton (1966) was used with modification. Before bone-marrow cells from femora were washed with 7 ml of isotonic NaCl solution, 0.03 ml of 0.03% colchicine solution was injected and kept for 1 hour. The cell suspension was centrifuged at 700 rpm for 8 minutes and resuspended in 7 ml of hypotonic solution (0.075 M KCl) for 22 minutes. Five ml of fixative (3 methanol: 1 acetic acid) were added and cells were spun down at 1,000 rpm for 10 minutes. The fixation-centrifuge sequences were repeated at least twice. The air-drying method by Rothfels and Siminovitsh (1957) was used for chromosomal preparation.

Slides were stained with 4% Giemsa solution (GIBCO) for 7 minutes, and rinsed with distilled water and air-dried. Good metaphases were photographed and printed so that the largest chromosomes were similar in size (about 1 cm). All acrocentric chromosomes were idiogrammed in order of decreasing length, and then metacentric chromosomes were done [chromosome nomenclature by Levan *et al.* (1964) was utilized]. Among five samples of *A. agrarius chejuensis* from Sanchondan the karyotype of one specimen (K-275) was only shown here because same results were obtained with other four specimens.

Phenetic analyses

Analyses were based on four external and 27 cranial characters as follows (for details see Koh, 1983): 1, greatest length of the skull; 2, condylobasal length; 3, length between incisor and incisive foramen; 4, length of the nasal bone; 5, zygomatic width; 6, mastoid width; 7, width of brain case; 8, height of brain case; 9, width between infraorbital canals; 10, length of rostrum; 11, length of hard palate; 12, interorbital constriction; 13, width across upper third molars; 14, incisor-upper-first-molar length; 15, width across upper first molars; 16, length of incisive foramen; 17, width of the interparietal bone; 18, length of the interparietal bone; 19, postpalatine length; 20, height of rostrum; 21, bullae-brain case height; 22, greatest length of mandible; 23, length of mandibular tooth row; 24, height of mandible; 25, length of ramus; 26, length of upper third molars; 27, length of upper first molars; 28, length of tail vertebrae; 29, length of hind foot; 30, body length; 31, length of ear.

Although sufficiently large samples were not available for each locality to be used as a basic unit, samples from the same locality were grouped as Operational Taxonomic Units, OTU's (see Table 2).

Sexual variation in striped field mice was not significant, whereas age variation was significant (Koh, 1983). Therefore, each specimen was assigned to one of five age classes (juvenile, subadult, young adult, middle-aged adult, and old adult) based on the eruption of upper third molar, degree of tooth-wear, and pelage colour (for details see Koh, 1983). Two hundred and ninety two samples were classified into 52 subadults, 91 young adults, 123 middle-aged adults, and 26 old adults (Table 2). The specimens of each adult age class were used for further analyses.

Table 2. Age classification of the samples of *Apodemus agrarius* from Korea. SA, YA, MA, and OA indicate subadult, young adult, middle-aged adult, and old adult, respectively.

Locality	OTU	Age classes				Total
		SA	YA	MA	OA	
Mosulpo	1	—	3	9	2	14
Jeju	2	—	3	3	—	6
Sanchondan	3	—	8	10	1	19
Youngsil	4	2	6	3	—	11
Saehwa	5	—	1	1	—	2
Jungmun	6	—	1	1	—	2
Bobhochon	7	—	1	—	—	1
Chongju	8	37	43	42	9	131
Mt. Weolak	9	6	4	13	3	26
Mt. Taebaek	10	2	6	8	3	19
Mt. Palgong	11	4	4	9	4	21
Mungyong	12	1	7	12	2	22
Anmyon Is.	13	—	2	4	—	6
Kunsan	14	—	1	6	1	8
Mokpo	15	—	1	2	1	4
Total		52	91	123	26	292

All computations were made using Chungbuk University HP-3000 computer. Sample statistics such as mean, standard deviation, and kurtosis were calculated by ELESTAT program of ISP, Interactive Statistical Programs.

For ordination analysis, principal component analysis (Seal, 1964), PCA, was performed from the individual measurements of 31 characters using subprogram PCAS of ISP. However, the data were singular in all analyses. Therefore, 13 characters (1, 3, 4, 6, 9, 13, 16, 19, 20, 22, 25, 28, and 30), showing significant difference among means with the four OTU's from *A. agrarius coreae* (see Koh, 1985), were selected and used for PCA (the means of 13 selected characters were also used and the measurements from OTU's with one sample were considered as the mean of each OTU). The results with middle-aged adults and young adults were shown here because same results were obtained with the data from old adults.

Discriminant analysis with the measurements of 31 characters was also carried out by DISCRIM of Statistical Packages for the Social Sciences, SPSS (Nie *et al.*, 1975). The result based on middle-aged adults were only shown here because same results were obtained even with the data from different age classes.

RESULTS

Multivariate analyses with individual measurements

Two dimensional configurations from PCA with the measurements of 13 selected characters of middle-aged adults are shown in Fig. 1 (numerals indicate OTU numbers). The correlations between original characters and the principal components are given in Tabel 3 (factors I, II, and III represented 49, 18, and 7 per cent of the variance, respectively). Two dimensional configurations from PCA with young adults are shown in Fig. 2 (factors I, II, and III represented 58, 8, and 7 per cent of the variance, respectively).

Two dimensional plotting from discriminant analysis with the measurements of 31 characters of middle-aged adults are shown in Fig. 3 (numerals indicate OTU numbers). The correlations between original characters and two canonical discriminant functions are given in Table 4 (functions I and II represented 46 and 14 per cent of the variance, respectively). The first discriminant axis scores of middle-aged adults are summarized as a histogram (Fig. 4).

The samples from seven OTU's (1, 2, 3, 4, 5, 6, and 7) were different from those of eight OTU's (8 to 15), as shown in Figs. 1, 2, 3, and 4. The highest coefficient in the first axis was shown in character 1 (greatest length of the skull), as shown in Tables 3 and 4.

Multivariate analyses with means

Two dimensional configurations from PCA with means of 13 selected characters in middle-aged adults are shown in Fig. 5 (numerals indicate OTU numbers). Factors I, II, and III represented 71, 9, and 8 per cent of the variance, respectively. Two dimensional configurations from PCA with the means of young adults are shown in Fig. 6 (factors I, II, and III represented 63, 11, and 8 per cent of the variance, respectively).

Seven OTU's (1, 2, 3, 4, 5, 6, and 7) formed one subgroup, whereas other eight OTU's (8 to 15) formed the other subgroup.

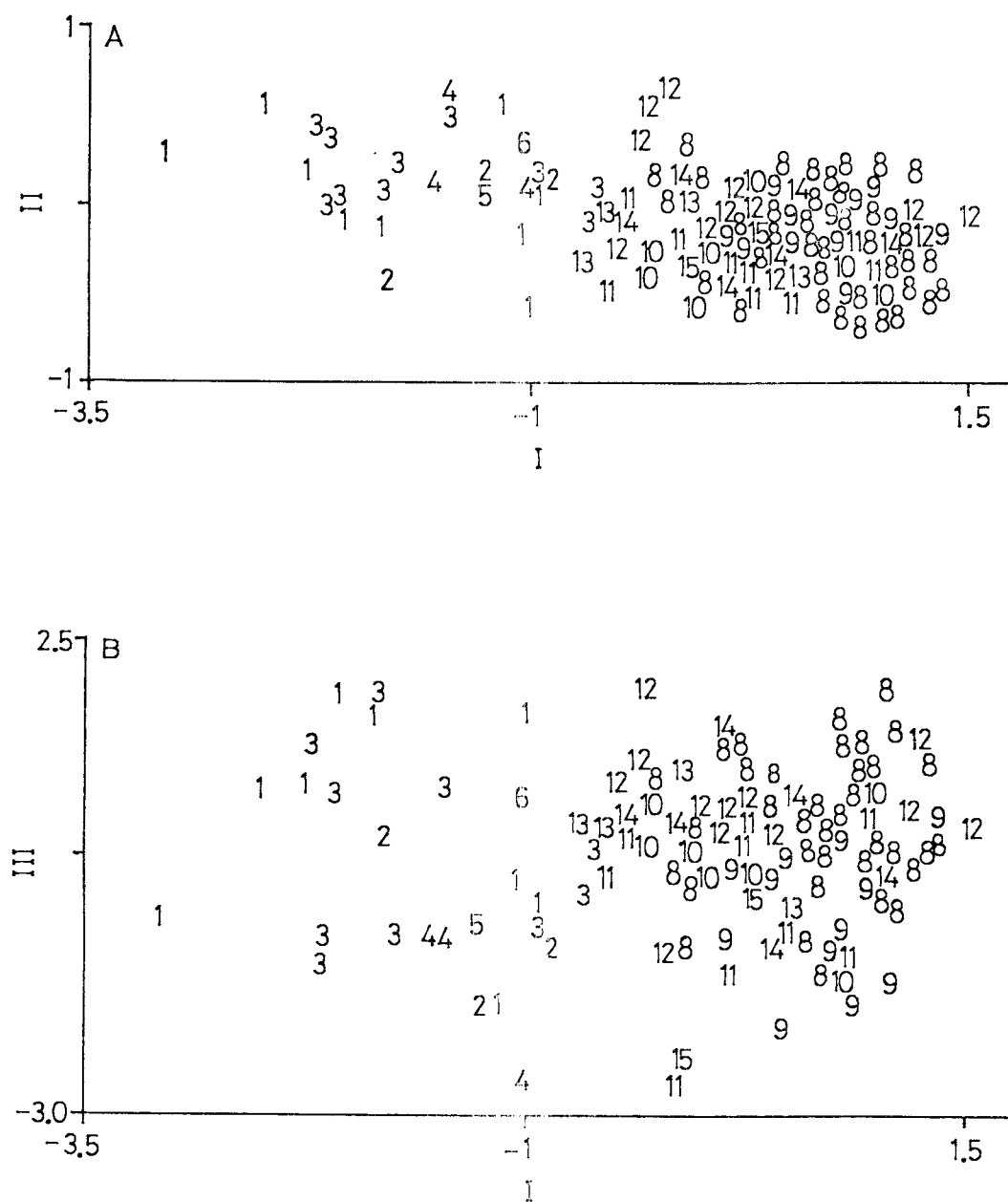


Fig. 1. Projections of middle-aged adults of *Apodemus agrarius* from Korea based on principal component analysis in three dimensions using 13 selected characters. Factors I, II, and III represented 49, 18, and 7 per cent of the variance, respectively. Numerals indicate OTU numbers. For locality and subspecies name of each OTU see Tables 1 and 2. A, samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III.

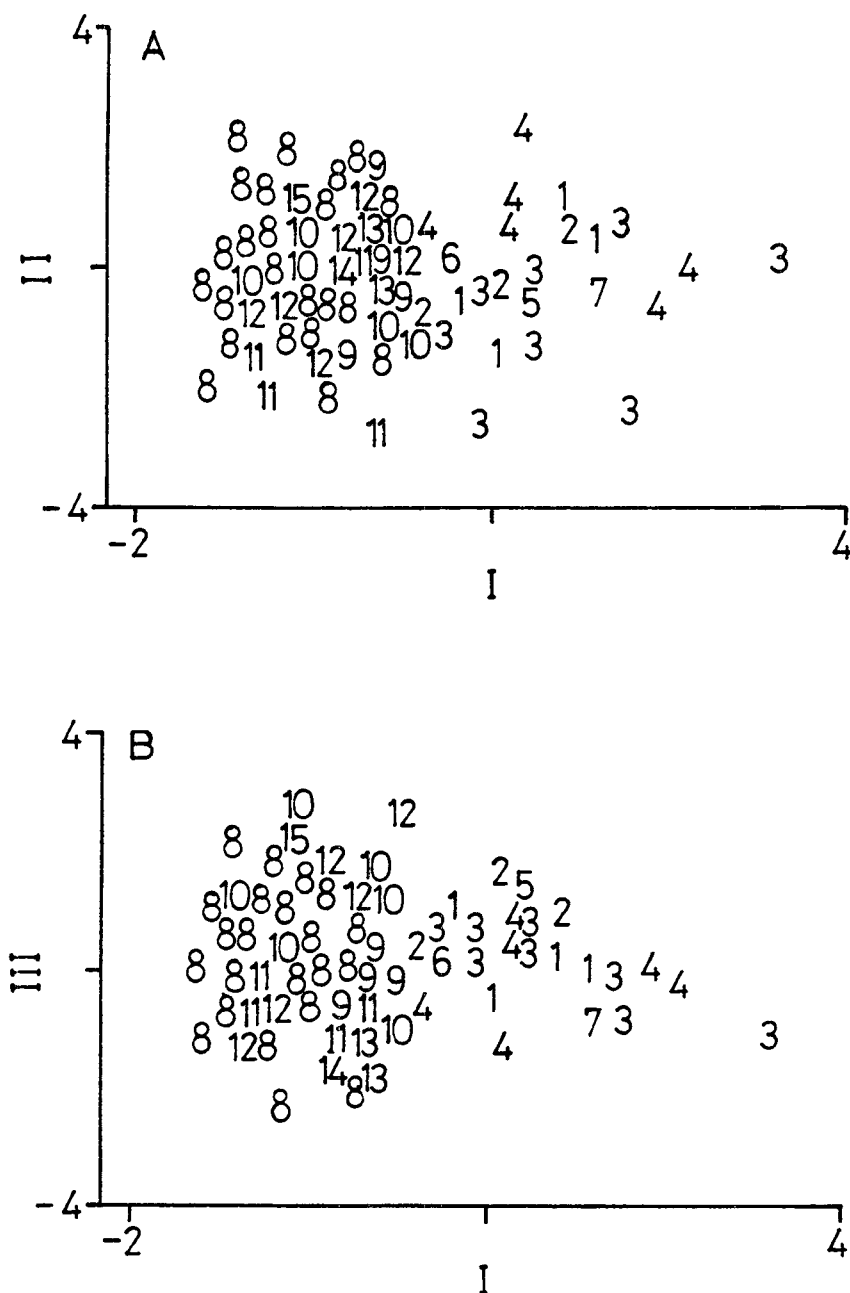


Fig. 2. Projections of young adults of *Apodemus agrarius* from Korea based on principal component analysis in three dimensions using 13 selected characters. Factors I, II, and III represented 58, 8, and 7 percent of the variance, respectively. Numerals indicate OTU numbers. For locality and subspecies name of each OTU see Tables 1 and 2. A, samples ordinated with factor I vs. factor II. B, samples ordinated with factor I vs. factor III.

Table 3. Principal components I, II, and III expressed as correlations between characters and individual components from an analysis of two subspecies of *Apodemus agrarius* (subspecies *chejuensis* and *coreae*). Individual measurements of 13 selected characters of 123 middle-aged adults were used.

Character	Factor I	Factor II	Factor III
1	-0.94	0.18	0.09
3	-0.80	0.14	0.06
4	-0.78	0.10	0.10
6	-0.74	0.19	-0.12
9	-0.61	0.03	-0.59
13	-0.62	-0.05	-0.48
16	-0.64	0.15	0.06
19	-0.90	0.16	0.11
20	-0.83	0.12	0.03
22	-0.56	-0.79	-0.01
25	0.08	0.97	0.03
28	-0.46	-0.77	0.15
30	-0.69	0.08	0.45
% of Trace	49	18	7

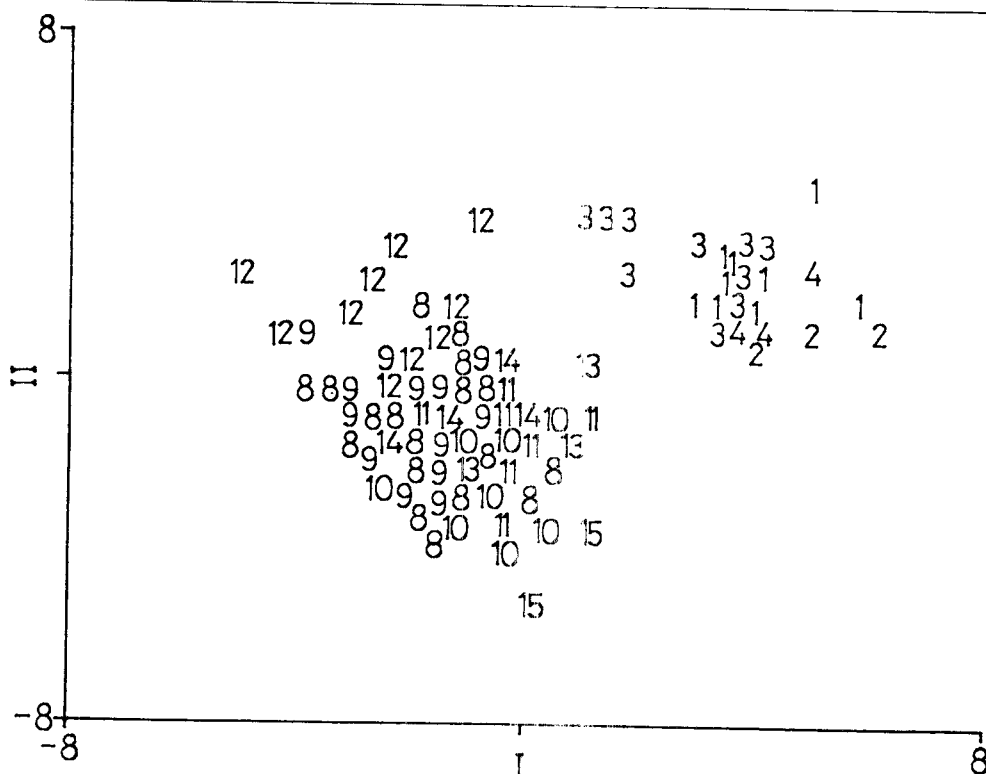


Fig. 3. Projections of middle-aged adults of *Apodemus agrarius* from Korea based on discriminant analysis in two dimensions using 31 characters. Functions I and II represented 46 and 14 percent of the variance, respectively. Numerals indicate OTU numbers. For locality and subspecies name of each OTU see Tables 1 and 2.

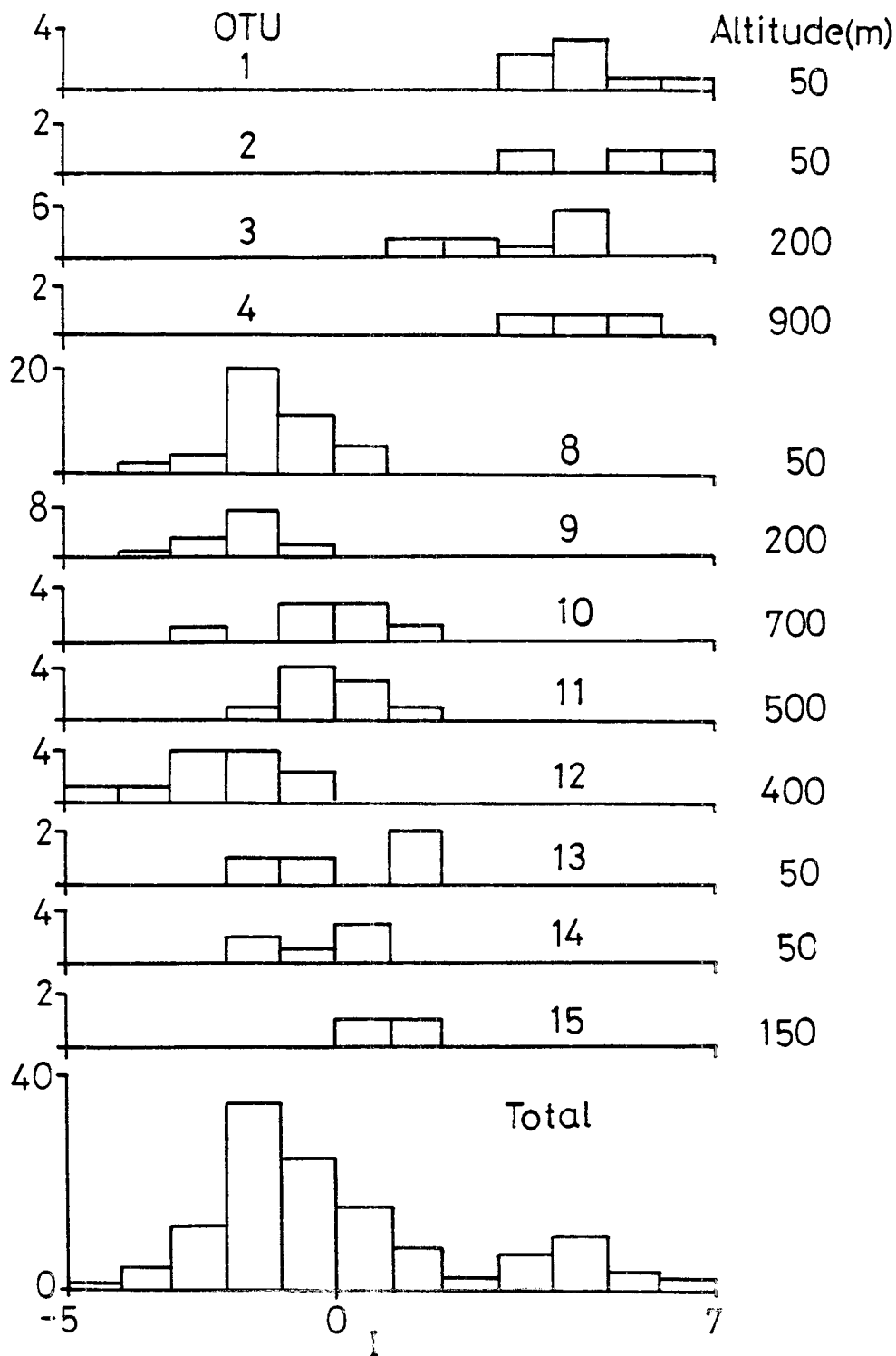


Fig. 4. Frequency distributions of the first discriminant axis scores based on the projections in Fig. 3. Thirty one characters of middle-aged adults of *Apodemus agrarius* from Korea were used. For locality and subspecies name of each OTU see Tables 1 and 2. Altitude of localities were obtained from Topographical maps (National Geography Institute, 1971).

Table 4. Discriminant functions I and II expressed as correlations between characters and individual functions from an analysis with two subspecies of *Apodemus agrarius* (subspecies *chejuensis* and *coreae*). The 31 characters of middle-aged adults were used.

Character	Function		Character	Function	
	I	II		I	II
1	0.72	-0.52	17	-0.06	0.43
2	0.40	-0.10	18	0.06	0.08
3	-0.19	-0.05	19	-0.68	0.37
4	-0.12	-0.09	20	0.14	-0.34
5	-0.22	0.72	21	0.16	-0.31
6	0.12	-0.12	22	0.35	-0.31
7	-0.13	0.18	23	0.29	-0.15
8	0.15	-0.12	24	-0.17	0.16
9	-0.14	-0.22	25	0.16	-0.14
10	-0.52	0.11	26	-0.03	-0.10
11	-0.32	0.10	27	0.09	-0.31
12	0.29	-0.29	28	-0.17	-0.58
13	0.22	-0.28	29	-0.12	0.60
14	0.57	0.49	30	-0.01	0.39
15	0.16	0.15	31	0.10	-0.01
16	-0.35	0.15			
% of Trace				46	14

Table 5. Summary of chromosomes counted in six samples of *Apodemus agrarius* (subspecies *chejuensis* from Sanchondan and subspecies *coreae* from Chongju).

Subspecies name	Specimen no.	Frequency of chromosome counted					2n
		46	47	48	49	50	
<i>A. agrarius chejuensis</i>	K-257	—	1	15	—	—	48
	K-259	1	1	12	1	—	48
	K-275	—	—	6	—	—	48
	K-0545	—	1	7	—	—	48
	K-270	—	1	13	1	1	48
<i>A. agrarius coreae</i>	K-271	1	1	31	—	1	48

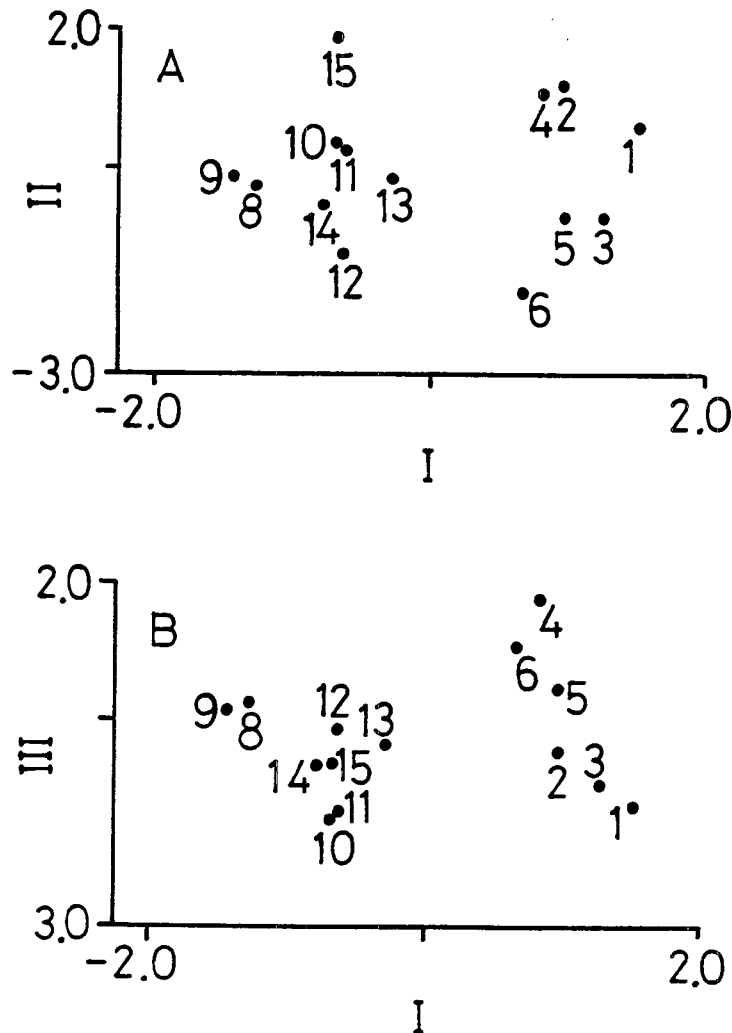


Fig. 5. Plottings of OTU's of *Apodemus agrarius* from Korea based on principal component analysis in three dimensions using means of 13 selected characters of middle-aged adults. Factors I, II, and III represented 71, 9, and 8 per cent of the variance, respectively. For locality and subspecies name of each OTU see Tables 1 and 2. A, OTU's ordinated with factor I vs. factor II. B, OTU's ordinated with factor I vs. factor III.

In summary, from the results of multivariate analyses shown above seven OTU's (1, 2, 3, 4, 5, 6, and 7) of *A. agrarius chejuensis* were larger than eight OTU's (8 to 15) of *A. agrarius coreae*. And character 1 (greatest length of the skull) seemed to be the most distinguishing character.

Chromosomal analyses

Diploid chromosome number and chromosome frequency of five samples of *A. agrarius chejuensis* from Sanchondan and one sample of *A. agrarius coreae* from Chongju are shown in Table 5. The diploid chromosome number of all samples was 48.

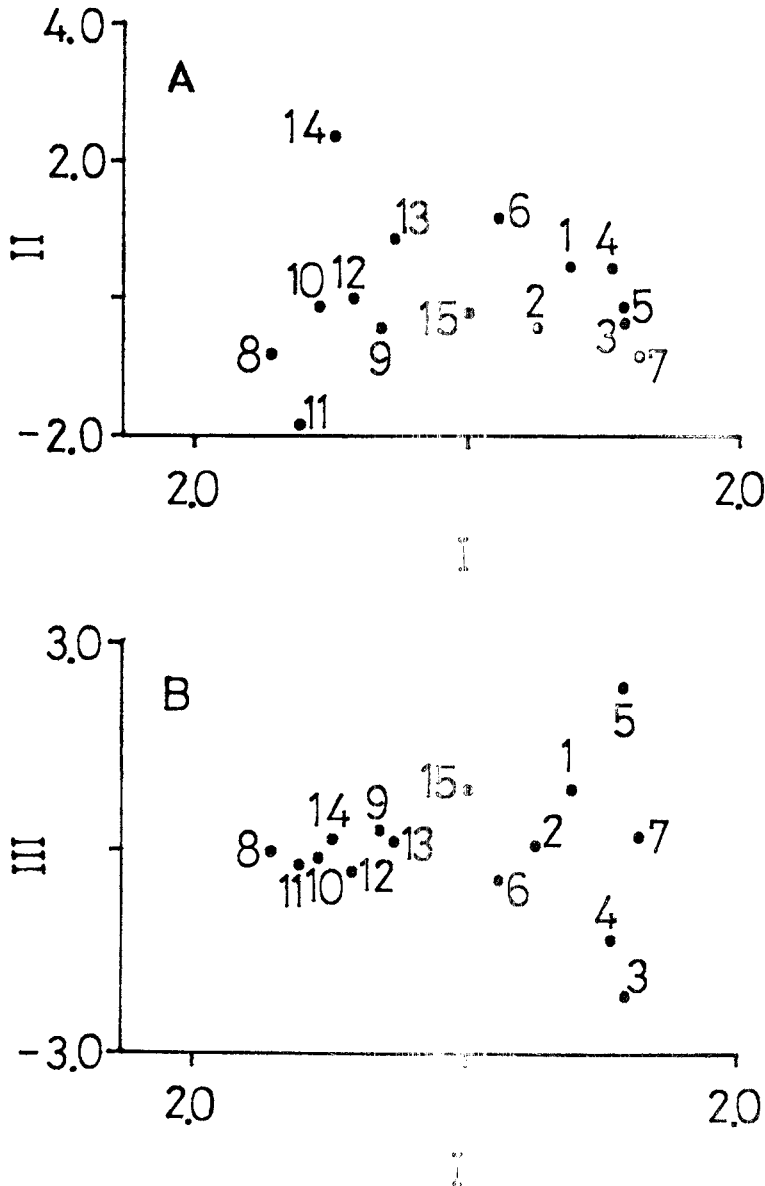


Fig. 6. Plottings of OTU's of *Apodemus agrarius* from Korea based on principal component analysis in three dimensions using means of 13 selected characters of young adults. Factors I, II, and III represented 63, 11, and 8 per cent of the variance, respectively. For locality and subspecies name of each OTU see Tables 1 and 2. A, OTU's ordinated with factor I vs. factor II. B, OTU's ordinated with factor I vs. factor III.

The karyotype of *A. agrarius coreae* from Chongju (K-270) is shown in Fig. 7. The representative karyotype of *A. agrarius chejuensis* from Sanchondan (K-275) is shown in Fig. 8. *A. agrarius coreae* from Chongju and *A. agrarius chejuensis* from Sanchondan are similar with each other in karyotype (40 acrocentric and 8 metacentric chromosomes).

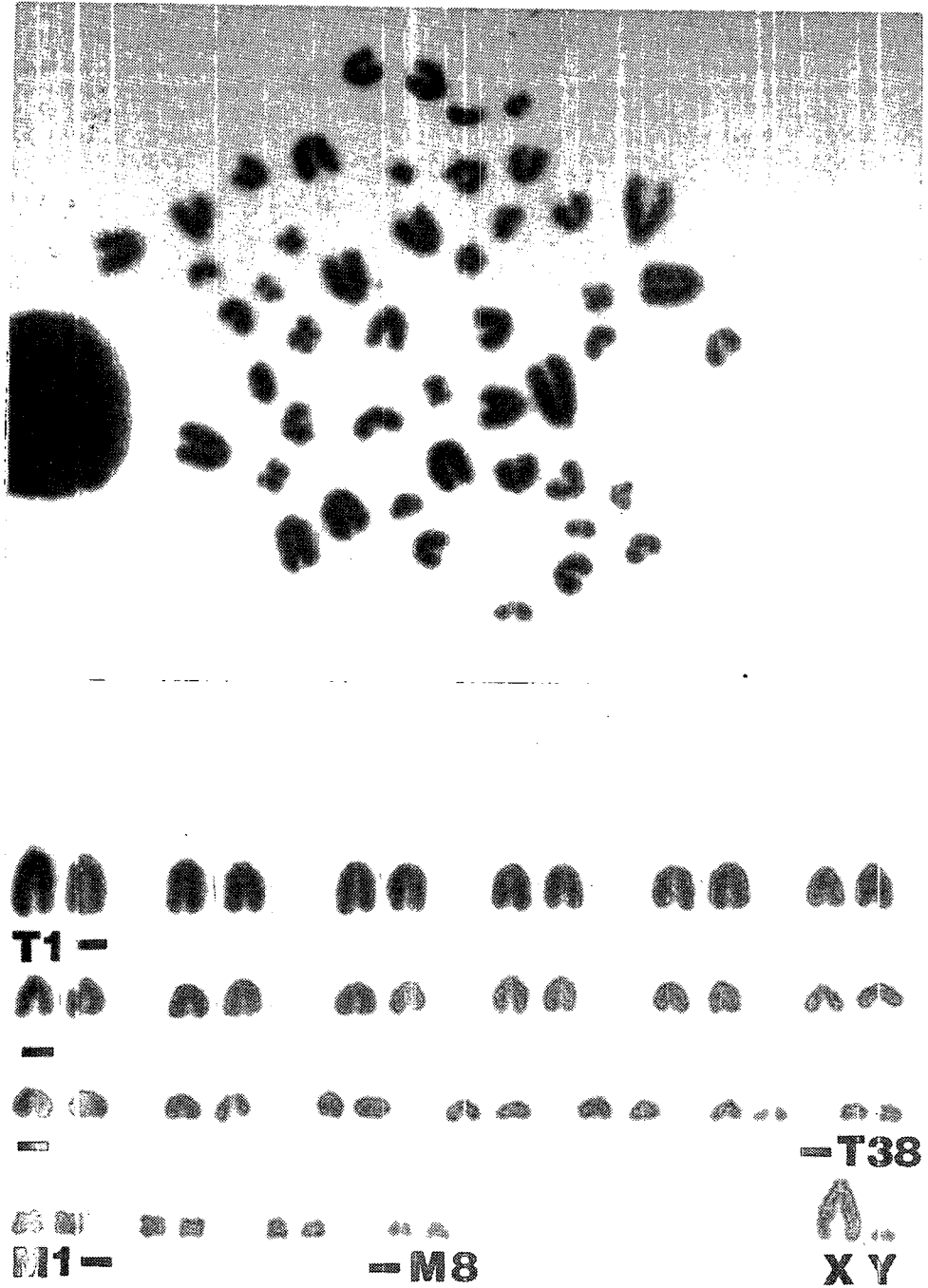


Fig. 7. Karyotype of a striped field mouse (K-270, ♂), *Apodemus agrarius coreac*, from Chongju. T and M indicate acrocentric and metacentric chromosomes, respectively.

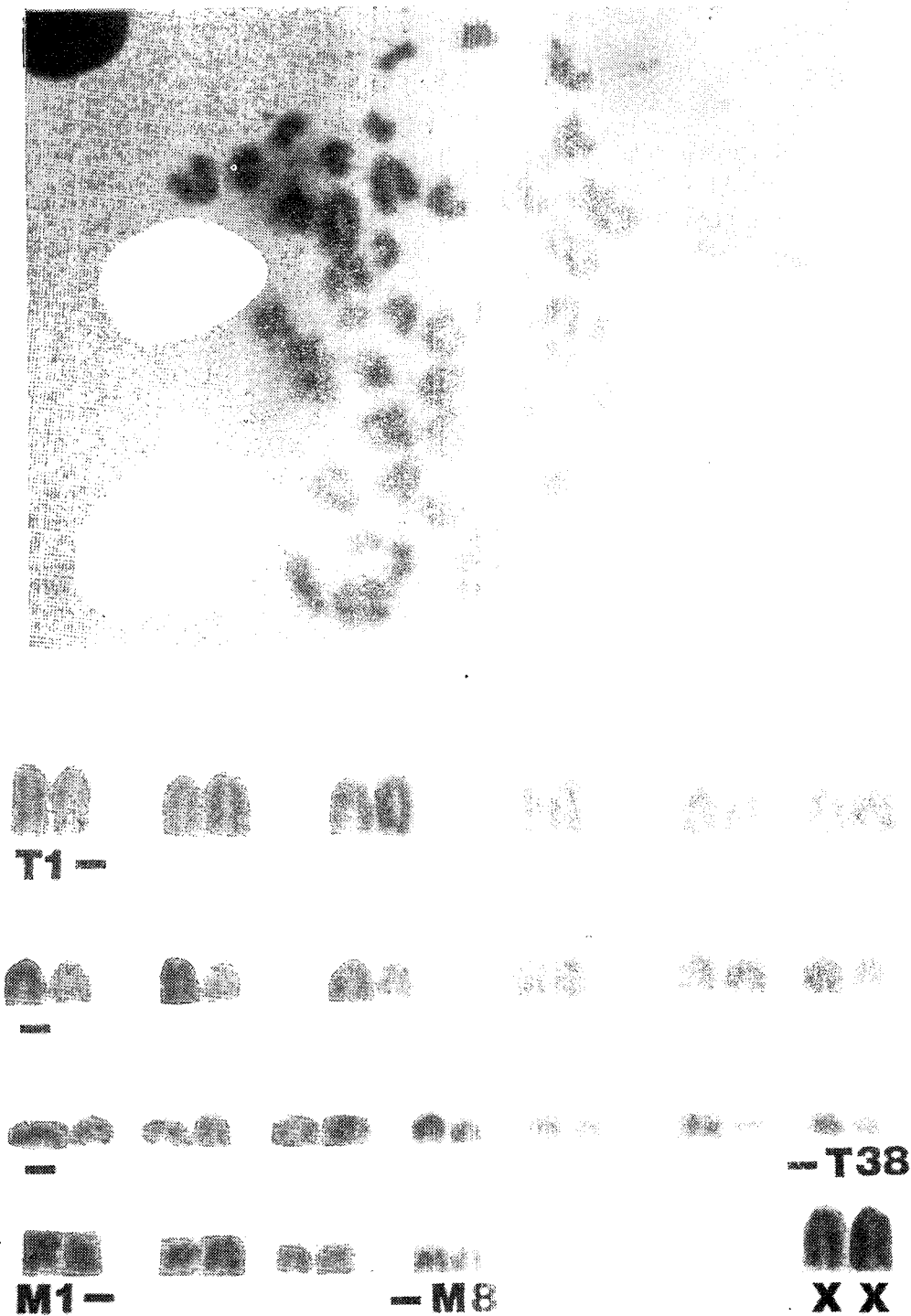


Fig. 8. Karyotype of a striped field mouse (K-275, ♀), *Apodemus agrarius chejuensis*, from Sanchondan. T and M indicate acrocentric and metacentric chromosomes, respectively.

DISCUSSION

Body size of individuals of different populations often negatively correlated with environmental temperature: such trend is sufficiently widespread to have been labeled to ecogeographical rules of Bergman (Brown and Lee, 1969). In the samples of *Apodemus agrarius coreae* from four localities clinal variation related with the altitude of localities was revealed in the first axis scores resulted from discriminant analysis and in the length of tail vertebrae (Koh, 1985).

In this study clinal variation was obscured when more specimens are included in the analysis of *Apodemus agrarius coreae*: moreover, this trend was not also revealed in *A. agrarius chejuensis* (see Fig. 4). Hayward (1965) noted that no correlation between body size and environmental temperature resulted from significant gene flow between the populations which might have counteracted much of the selection for divergence in body size.

It was found that *Apodemus agrarius coreae* and *A. agrarius chejuensis* are similar in karyotypes (see Figs. 7 and 8). And it was confirmed that *A. agrarius chejuensis* is a large-size group and *A. agrarius coreae* is a small-size group (Figs. 1 to 6). Wilson *et al.* (1975) noted that in mammalian phylogenies the rates of anatomical and chromosomal evolution are rather close. Moreover, Bykova *et al.* (1978) found that Asian mountain vole, *Alticola lemnus*, from Yukutia and Chukotka are appreciably different in karyotypes and morphology. Dobzhansky (1972), however, noted that genetic divergence and speciation may occur without a rearrangement of the genetic materials in the chromosomes, although more often the two processes go hand in hand.

Geographically isolated populations may be either species or subspecies (Wiley, 1982) and it is preferable for various reasons to treat allopatric populations of doubtful rank as subspecies (Mayr, 1969). Crowson (1970) noted that a discontinuity in characters of structure will normally show when members of two different species are compared. Jardin and Sibson (1971) stated that the distinct criteria for populations to be recognized as different species are: a) dissimilarity in morphological attributes; b) differences in ecological and/or geographical range; c) degree of interfertility; d) cytological differences; and e) differences of enzyme and proteins. Ross (1974) also stated that when there is no intermediate between two parapatric groups they are different species.

Jeju island (Jeju-Do) was formed by series of volcanic activities at the end of Tertiary period in Cenozoic era (Park, 1985) and in recent it was 20,000 years since this island had been isolated from the Korean peninsula by the increase of sea level (Seyfert and Sirkin, 1984). Moreover, such islands as Wan, Chuja, and Jin islands connect Jeju island to the Korean mainland: these islands can be sweepstake route of dispersal, as stated by Simpson (1940) and they might be inhabited by founders (Mayr, 1982) of two size groups, small-size group of *A. agrarius coreae* and large-size group of *A. agrarius chejuensis*.

In future, specimens of striped field mice from southwestern regions of the Korean peninsula, including samples from those islands mentioned above, are needed for phenetic, electrophoretic, and chromosomal studies in order to clarify the subspecific status of *A. agrarius chejuensis*. Moreover, it is necessary to collect specimens of Korean field mice (*A. peninsulae peninsulae*), which is the other species of the genus *Apodemus* in Korea, in order to compare with samples of striped field mice from Korea.

ABSTRACT

Striped field mice from Jeju-Do (*Apodemus agrarius chejuensis*) were used for morphometric and chromosomal studies in order to compare them with striped field mice from the Korean peninsula (*A. agrarius coreae*). It was found that *A. agrarius chejuensis* and *A. agrarius coreae* are similar in karyotypes and it was confirmed that the former is a large-size group and the latter is a small-size group.

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